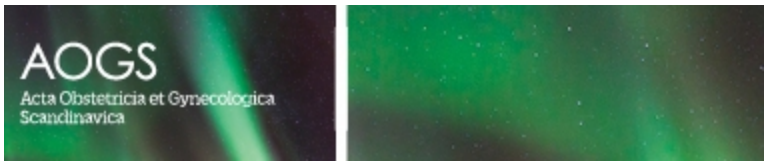


# Plasma long-chain omega-3 fatty acid status and risk of recurrent early spontaneous preterm birth: a prospective observational study

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|---|---|
| Journal: | *Acta Obstetricia et Gynecologica Scandinavica* |
| Manuscript ID | AOGS-20-1477.R2 |
| Wiley - Manuscript type: | Original Research Article |
| Date Submitted by the Author: | 12-Mar-2021 |
| Complete List of Authors: | Goodfellow, Laura; University of Liverpool, Harris-Wellbeing Research Centre; Liverpool Women's Hospital NHS Foundation Trust,<br>Care, Angharad; University of Liverpool, Harris-Wellbeing Research Centre<br>Harrold, Jane; University of Liverpool, Harris-Wellbeing Research Centre<br>Sharp, Andrew; University of Liverpool, Women's and Children's Health<br>Ivandic, Jelena; Liverpool Women's Hospital NHS Foundation Trust,<br>Poljak, Borna; Liverpool Women's Hospital NHS Foundation Trust,<br>Roberts, Devender; Liverpool Women's Hospital NHS Foundation Trust,<br>Alfirevic, Ana; University of Liverpool, Harris-Wellbeing Research Centre; University of Liverpool, Department of Pharmacology and Therapeutics<br>Muller-Myhsok, Bertram; University of Liverpool, Harris-Wellbeing Research Centre; Max-Planck-Gesellschaft, Department of Translational Research in Psychiatry<br>Gibson, Robert; The University of Adelaide, School of Agriculture, Food and Wine<br>Makrides, Maria; South Australian Health and Medical Research Institute<br>Alfirevic, Zarko; University of Liverpool, Harris-Wellbeing Research Centre; Liverpool Women's Hospital NHS Foundation Trust, |
| Keywords: | Preterm Birth |
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# Plasma long-chain omega-3 fatty acid status and risk of recurrent early spontaneous preterm birth: a prospective observational study


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## Conflict of Interest statement

All authors have completed the Unified Competing Interest form and declare: LG has received study support grants from Wellbeing of Women charity for this research; AC has received salary and study support grants from Wellbeing of Women charity for this research; JH, AS, JI, AA, BM-M and ZA were also members of the University of Liverpool during the grant from Wellbeing of Women; MM and RG received a Centre of Research Excellence grant from the Australian National Health and Medical Research Council for this work; MM and RG have served on the board of Trajan Nutrition within the past three years; RG holds a patent on stabilizing and analysing fatty acids; no other financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

## Funding statement

The prospective cohort study was funded as part of a charitable donation that founded the Harris-Wellbeing Preterm Birth Research Centre, University of Liverpool. This was awarded by external peer review including patient representatives. The charitable donation covered administrative costs, laboratory costs within the University of Liverpool, salary for AC, and study support costs for AC and LG. The laboratory analysis within the South Australian Health and Medical Research Institute (SAHMRI) unit was funded by a Centre of Research Excellence grant from the Australian National Health and Medical Research Council (1135155), with external peer review. The funders had no role in the analyses, interpretation of results, or the writing of this manuscript.

## Structured abstract

### Introduction

A 2018 Cochrane review found that omega-3 supplementation in pregnancy was associated with a risk reduction of early preterm birth of 0.58; prompting calls for universal supplementation. Recent analysis suggests the benefit may be confined to women with a low baseline omega-3 fatty acid status, however the contemporary UK pregnant omega-3 fatty acid status is largely unknown. This is particularly pertinent for women with a previous preterm birth, in whom a small relative risk reduction would have a larger reduction of absolute risk.

This study aimed to assess the omega-3 fatty acid status of a UK pregnant population and determine the association between the long-chain omega-3 fatty acids and recurrent spontaneous early preterm birth.

### Materials and Methods

283 high-risk women with previous early preterm birth were recruited to the prospective observational study in Liverpool, UK. Additionally, 96 pregnant women with previous term births and birth $\geq 39^{+0}$ weeks in the index pregnancy provided a low-risk population sample.

Within the high-risk group we assessed the odds ratio of recurrent early preterm birth compared to birth at $\geq 37^{+0}$ weeks gestation according to plasma eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) at 15-22 weeks gestation.

### Results

Our participants had low EPA+DHA; 62% (143/229) of women with previous PTB and 69% (68/96) of the population sample had levels within the lowest two quintiles of a previously published pregnancy cohort.

We found no association between long-chain omega-3 status and recurrent early preterm birth (n=51). The crude odds ratio of a recurrent event was 0.91 (95% CI 0.38 to 2.15, p=0.83) for women in the lowest, compared to the highest three quintiles of EPA+DHA.

## Conclusions

In the majority of our participants levels of long-chain omega-3 were low; within the range that may benefit from supplementation. However, levels showed no association with risk of recurrent early sPTB. This could be because our population levels were too low to show benefit in being omega-3 'replete'; or else omega-3 levels may be of lesser importance in recurrent early preterm birth.

## Keywords

Preterm birth, omega-3, long-chain polyunsaturated fatty acids

## Abbreviations

BMI: Body mass index

DHA: Docosahexaenoic acid

EPA: Eicosapentaenoic acid

IMD: Index of multiple deprivation

LLETZ: Large Loop Excision of Transformation Zone of cervix

PPROM: Preterm Prelabour Rupture of Membranes

sPTB: Spontaneous preterm birth

## Key message

UK pregnant women have low omega-3, whether they have had a previous preterm birth, or not. Surprisingly levels don't relate to recurrent early preterm birth risk. Should we supplement?

# MAIN TEXT

## INTRODUCTION

Globally preterm birth is the leading cause of death in children under 5 years old.[1] Previous preterm birth is the strongest risk factor for subsequent preterm delivery.[2] A 2018 Cochrane review concluded that omega-3 supplementation was an effective strategy to prevent preterm birth, with a 42% risk reduction (from 46 to 27 per 1000 births; 95% CI, 23-56) for preterm birth less than 34 weeks.[3] A subsequent randomised controlled trial[4] with secondary analysis[5] suggested the benefit may be confined to women with a low baseline total long chain omega-3 fatty acid level. Worryingly, within the secondary analysis[5] supplementing women with higher total long chain omega-3 fatty acid status was associated with increased rates of early preterm birth.

The fatty acid components with the strongest evidence of preterm birth prevention are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA),[3] which are collectively referred to as long-chain omega-3 fatty acids. These nutrients are predominantly obtained from oily fish and seafood and associated with a more affluent diet. The long-chain omega-3 intake in pregnancy in the UK has been estimated from food frequency questionnaires as low,[6,7] or adequate[8] in three studies between 1991 and 2007.

Liverpool Women's Hospital has a tertiary referral preterm birth prevention clinic that serves the 4th most deprived local authority area in England (out of 343).[9] Based on the Cochrane review[3] findings we offered omega-3 supplementation to these high risk women from February 2019.[10] However, we were unsure whether this would offer benefit because of the unknown baseline long-chain omega-3 status in our population. Plasma levels of omega-3 in the UK pregnant population have not been assessed to our knowledge. The Danish National Birth Cohort[11,12] showed that women in the lowest quintile of plasma EPA+DHA (<1.42% of total fatty acids), in the second trimester, had a 2.13 times increased risk (95% CI 1.18-3.79) of sPTB under 34 weeks compared to women in quintiles 3-5. The association between omega-3 and preterm birth was not present with levels in the third quintile and above. This is consistent with Simmonds *et al*[5] and suggests that the main benefit of supplementation is in pregnancies with a lower baseline long-chain omega-3 status.

Importantly there has been a recent corrigendum[12] to the original research within the Danish National Birth cohort[11] based on the effect of thawing of stored samples prior to analysis of long-chain omega-3 fatty acids; this was therefore addressed within our analysis too.

We had two objectives. Firstly, to determine the expected distribution of long-chain omega-3 fatty acids within 'healthy pregnancies' in our locality; low-risk pregnant women who delivered at ≥39 weeks without preterm prelabour rupture of membranes (PPROM). Secondly, to assess the relationship between long-chain omega-3 status and recurrent early (under $34^{+0}$ weeks) spontaneous preterm birth (sPTB) and PPROM in our region.

## MATERIALS AND METHODS

Women with singleton pregnancies were enrolled at Liverpool Women's Hospital from 1st April 2012 until 31st December 2017 as part of "The development of novel biomarkers for prediction of preterm labour in a high-risk population study". Participants were invited to two visits at approximately 16 ($15^{+1}$-$18^{+6}$ weeks) and 20 weeks gestation ($19^{+0}$-$23^{+0}$). For the purposes of this analysis the first sample available was used (single sample per participant).

A flowchart of selection entry from two different obstetric populations is shown in Figure 1. A 'high-risk' population consisted of women with a history of sPTB or PPROM at $16^{+0}$-$33^{+6}$ weeks gestation. Low-risk women were parous women with all previous births ≥$37^{+0}$ weeks gestation. Full details of the recruitment process, inclusion criteria and careful pregnancy outcome classification criteria are given in Appendix A. Participants were excluded from the statistical analysis if omega-3 supplements had been used in pregnancy.

To describe the expected distribution of omega-3 fatty acids in our population low-risk women that delivered ≥$39^{+0}$ weeks were selected (low-risk population sample).

Recurrent early sPTB/PPROM was defined as high-risk participants who had a late miscarriage, PPROM or sPTB at $16^{+0}$-$33^{+6}$ weeks gestation. High-risk women who gave birth ≥$37^{+0}$ weeks gestation without PPROM were allocated to the high-risk term birth group.

## Omega-3 fatty acid analysis

Maternal blood samples were taken in 10ml BD vacutainer® tubes containing K2EDTA (dipotassium ethylenediaminetetraacetic acid), placed on ice immediately and processed within 1 hour of sampling. Tubes were centrifuged at 3000rpm for 10mins at 4°C. Plasma was aspirated and stored in cryovials at -80°C. A total of 30uL was transferred to blood spot cards that were coated in antioxidants and chelating agents so as to minimise oxidation of polyunsaturated fatty acids.[13] The dried blood spot cards transported by post to SAHMRI (South Australian Health and Medical Research Institute) where the plasma spots were transesterified and distributions of fatty acids were determined by capillary gas chromatography.[13] The laboratory team were blinded to the pregnancy status of the samples.

## Statistical analysis

Statistical analysis was performed in Stata version 15.1. The distribution of long-chain omega-3 fatty acids within the low-risk population sample were calculated and used to define quintiles of total omega-3, DHA and EPA for our population.

Histograms were used to show the distribution of long-chain omega-3 fatty acids within the high-risk group according to whether the participant did, or didn't, have recurrent early sPTB/PPROM. Two-term fractional polynomials were then used to visualise the expected non-linear association between long-chain omega-3 fatty acids levels and risk of recurrent early sPTB/PPROM within the high-risk group.

High-risk participants in the early sPTB/PPROM and high-risk term birth groups were assigned to the quintiles based on the distribution of total omega-3, DHA and EPA within the low-risk population sample and to the quintiles described by Olsen *et al.*[11,12] Binomial logistic regression was used to calculate the odds ratios of early sPTB/PPROM compared to term birth per quintile. Quintiles 3-5 were combined and used as the reference group based on previous work.[11,12] Analysis was performed unadjusted and adjusted for covariates that were selected based on biological plausibility. The chosen covariates were: maternal age at study participation; maternal body mass index (BMI); maternal smoking at time of study visit (binary outcome of yes/no); and index of multiple deprivation (IMD). Age and BMI were converted to quadratic terms because of the bimodal relationships between these variables and risk of preterm birth. IMD was obtained using the woman's home postcode on the UK government website.[14] The IMD ranks every neighbourhood in England from 1 (most

deprived) to 32844 (least deprived).[9] The IMD is a collective score summarising income deprivation, employment deprivation, health deprivation and disability, education skills and training deprivation, barriers to housing and services, living environment deprivation, and crime. IMD scores were used as continuous variables within the logistic regression.

Adjusted odds ratios for early sPTB/PPROM are presented both for the participants with all co-variates available, and for all participants using imputation for missing co-variates. Multiple imputation using chain equations was used to account for missing data as this allows for binary covariates (such as smoking). The proportion of total sampling variance due to missing data for IMD was 48%, therefore as recommended 50 imputations were performed.[15] The variables used in the imputation model were all of the covariates described above as well as: pregnancy outcome (birth at term or early sPTB/PPROM); quintile of total omega-3, EPA, DHA and DHA plus EPA; and quintile according to Olsen *et al.*[11,12] No auxiliary variables were identified.

During the course of this work concern was raised that prior thawing may alter plasma long-chain omega-3 fatty acid analysis.[12] We therefore undertook three further pieces of statistical analysis. Firstly, assessment was performed of the long-chain omega-3 fatty acid status by number of prior freeze-thaw cycles of the sample. Secondly the number of freeze thaw cycles was included as a covariate in the logistic regression described above. Finally the binomial logistic regression was repeated using only samples that had undergone prior freeze-thaw cycles, and those without.

### Ethical approval

The study was approved by North West Research Ethics Committee- Liverpool Central, reference 11/NW/0720 on 4th November 2011.

## RESULTS

We recruited 296 high-risk participants and 271 low-risk participants. Of 283 high-risk women with data suitable for analysis, 51 (18%) had a recurrent early sPTB or PPROM and 178 (63%) had term births (≥37weeks) without PPROM (Figure 1). Of 271 low-risk participants, 188 gave birth at $\geq 39^{+0}$ weeks without PPROM, and had samples suitable for

analysis. We selected the first 100 of these participants to send samples for laboratory analysis. Four of these participants were subsequently noted to have used omega-3 supplementation, and so the remaining 96 participants formed the low-risk population sample.

The baseline characteristics and pregnancy outcomes are broadly similar across the pregnancy groups (Table 1), except for known risk factors for sPTB/PPROM. Compared to the low-risk population sample, more of the high-risk participants smoked (9.7% of low-risk vs 24.0% of high-risk group), and the high-risk participants had slightly lower IMD scores (more social deprivation). Preterm birth prevention treatment was offered in accordance with UK national guidelines.[16] None of the low-risk women required an intervention but 32.8% (93/283) of the high-risk women did.

The low-risk population sample were used to define the expected distribution of omega-3 fatty acid levels in our population (Table 2).

Levels of total omega-3, DHA and EPA within the high-risk group show similar distributions in women who have an early sPTB/PPROM, and those who do not (Figure 2 A-D). The risk of recurrent early sPTB by total omega-3, DHA and EPA levels are visualised in Figure 2 E-H. Visually, it appears there could be a weak relationship between higher levels of EPA, DHA and total omega-3 and preterm birth, but the wide confidence intervals are also consistent with no correlation.

The high-risk group was then split according to total omega-3, DHA and EPA quintiles obtained from low-risk population sample into three groups: quintile 1, 2 and 3-5 (reference group) (Table 3). When pregnancy outcomes were compared between quintile groups, the early sPTB/ PPROM rate was lower in quintiles 1 and 2 for total omega-3 (crude OR 0.65, 95% CI 0.23-1.84 and 0.52, 95% CI 0.23-1.15, respectively), EPA plus DHA, DHA and EPA, although none of these differences reached conventional statistical significance ($p<0.05$) (Table 3).

We performed the same analysis adjusting for covariates of smoking, maternal age, BMI, and IMD, both restricting the analysis to participants with all variables available and using multiple imputation to account for missing variables (Table 3). These results also showed no association between long-chain omega-3 fatty acids and early sPTB/PPROM, and the non-significant trend towards higher risk of preterm birth with higher levels.

Omega-3 fatty acid levels were universally lower in our population than the Danish National Birth Cohort[11,12] (Table 4). 66% (63/96) of our low-risk population sample had plasma DHA+EPA levels within the lowest two quintiles of the Danish cohort (compared to the expected 40%). Levels within the lowest two quintiles of the Danish cohort were also found in 51% (26/51) of high-risk women who had recurrent early sPTB/PPROM and 56% (100/178) of high-risk women who had term births. Unadjusted and adjusted analyses also showed no association between EPA plus DHA levels and early sPTB/PPROM using the Olsen *et al.*[11,12] quintiles (Table 4).

Prior to our analysis samples from 17% (16/96) of our low-risk population sample, 9.0% (16/178) of our high-risk term birth group and 57% (29/51) of our high-risk early sPTB/PPROM groups had undergone three freeze thaw cycles (Table S1). The remainder of samples had undergone no prior freeze:thaw cycles. We found no statistically significant difference in DHA, EPA or DHA+EPA levels when comparing samples with and without prior freeze thaw cycles, but within the high-risk reference group there was a trend for slightly lower omega-3 fatty-acid levels in samples that had undergone prior freeze-thaw cycles. When the logistic regression described in Table 3 was repeated in samples both without prior freeze:thaw cycles (Table S2) and with prior freeze: thaw cycles (Table S3) there remained a non-significant trend towards a reduced chance of sPTB/PPROM with lower omega-3 fatty-acid levels in both analyses.

## DISCUSSION

Contrary to the previous findings, we did not demonstrate a relationship between long-chain omega-3 levels and spontaneous preterm birth. This was despite comparing plasma total omega-3, DHA and EPA levels to both 'healthy' pregnancies in our population, and to levels in Danish pregnant women that have previously been associated with preterm birth.[11,12] In our population, both women at high and low risk of preterm birth had lower levels of plasma DHA plus EPA than those described in the Danish population.[11,12]

The plasma long-chain omega-3 levels within our population could have been so low that we did not have enough 'replete' participants to show the benefit in preterm birth reduction with adequate levels. However, our results show a non-significant trend in the opposite direction

to previous literature (i.e. a higher risk of preterm birth with a higher level of omega-3, DHA and EPA), and no biological gradient.

Women with a previous preterm birth are often highly motivated to avoid recurrence, and could have become aware of evidence to support increased omega-3 intake[17,18] during their pregnancy. Omega-3 fatty acids may have a rapid effect on risk of preterm birth.[19,20] If a substantial number of the women in our study actually did increase their omega-3 fatty acid intake during pregnancy, this may have confused the relationship between omega-3 measured in early 2nd trimester and subsequent risk of early preterm birth.

To our knowledge this is the fourth analysis relating blood DHA and EPA levels in the second trimester to preterm birth risk. Previous studies include the analysis by Olsen *et al.,*[11] and the secondary analysis of the ORIP trial,[5] both demonstrating lower long-chain omega-3 levels in association with preterm birth under 34 weeks, in Danish and Australian populations respectively. The third study is a secondary analysis of a trial of omega-3 supplements to prevent recurrent preterm birth under 37 weeks in the US.[21] Klebanoff *et al.* did find that participants in the lowest quartile of DHA+EPA had a higher rate of preterm birth (83/176, 47.2%) compared to the highest quartile (63/175, 36%), however their results also did not reach statistical significance.[21]

A strength of this study is that our preterm group included only recurrent sPTB, or PPROM, before $34^{+0}$ weeks gestation. We aimed to achieve as pure 'phenotype' of spontaneous preterm birth as possible. Previous studies into the association between omega-3 levels and preterm birth have included all births under 34 weeks,[5] or 37 weeks,[21] or only excluded cases of preeclampsia prior to 34 weeks.[11] It is possible that the benefit of omega-3 to prevent preterm birth is confined to medically indicated preterm birth from conditions such as preeclampsia and growth restriction.[22] However, the most recent Cochrane review shows no impact of omega-3 supplementation upon these conditions.[3] In keeping with this our initial visualisation of the relationship between long-chain omega-3 status and early preterm birth in the whole high-risk group, including those with late medically indicated preterm births (Figure 2), did not show an association between long-chain omega-3 levels and all preterm births. Alternatively the impact of omega-3 upon preterm birth prevention may be within the low-risk population, that was not assessed for preterm birth risk in this study.

A limitation of this study is that 56% of sPTB/PPROM samples had undergone prior freeze:thaw cycles, in comparison to only 9% of the high-risk reference group. However, we found higher than expected levels of omega-3 fatty-acids in the sPTB/PPROM group, and freeze:thaw cycles might be expected to lower the expected levels of omega-3 fatty-acids.[12] We therefore do not feel that this has materially impacted upon our findings.

We acknowledge that plasma levels of omega-3, DHA and EPA were measured on samples from participants that were not fasted, however, the previous study finding an association between plasma levels of DHA and EPA and preterm birth used samples taken by GPs at routine visits and no mention is made of fasting in the description.[11,23]

This was a pragmatic study based on a biomarker study that had finished recruiting at the time of study inception. As such no formal power calculation has been performed, and we did not have a pre-defined *a priori* level at which we are able to accept/reject our null hypothesis of no association between long-chain omega-3 levels and recurrent spontaneous early preterm birth. Nevertheless, we feel that knowledge of the low baseline levels of long-chain omega-3 fatty acids within pregnant women in the UK, and also no indication of an association between long-chain omega-3 fatty acids and recurrent preterm birth in our high-risk group is important to inform the discussion about omega-3 supplementation for preterm birth prevention.

It is possible that preterm birth prevention therapy averted preterm birth in some high-risk participants, attenuating an association between omega-3 and early sPTB/PPROM. Analysis limited to participants without preterm birth prevention treatment showed similar findings (data not shown). Any intervention involving omega-3 is likely to be applied in combination with current treatments, and so we felt it was optimal to assess the situation within current clinical practice.

Preterm birth is a multifactorial disease and the contribution of a single factor (such as omega-3 levels) is likely to only be modest. It is possible that other factors, genetic or environmental, leading to recurrent preterm births are able to ‘overpower’ any contribution of long-chain omega-3 status. We suggest that future research should include baseline long-chain omega-3 fatty acids testing on a large scale, and evaluate the influence of these levels on other risk factors of preterm birth. This would be relevant to both women with, and

without, identifiable risk factors for preterm birth, and may be achieved by an individual patient data meta-analysis of already conducted work.

## CONCLUSION

We found low plasma omega-3, DHA and EPA levels in the second trimester in women at high and low-risk of preterm birth. The previously described association between low DHA and EPA and preterm birth was not replicated. We suggest that either plasma long-chain omega-3 fatty acids were so low in this population we didn't have enough 'replete' participants to show a benefit, or there are alternative mechanisms for recurrent early preterm birth in this setting.

### *Acknowledgements*

We would like to thank all participants for their enthusiastic involvement in the study, in particular members of the Harris-Wellbeing Patient and Public Engagement group. We would also like to thank Mrs Tracy Ricketts for administrative support with the study, and the Liverpool Women's Hospital for hosting the research.

### *Tweetable Abstract*

UK pregnant women have low omega3. Surprisingly levels don't relate to recurrent early preterm birth risk. Should we supplement? @DrLGoodfellow @Angharad84 @asharpliverpool @WellbeingHarris

### Contributors

AC, AS, DR, BM-M, AA and ZA conceived the study, wrote the protocol and obtained funding. AC, JI, BP and LG contributed to the protocol, recruited participants and performed the initial laboratory analysis. JH managed the samples and oversaw the Liverpool component of the laboratory analysis. RG oversaw the SAHMRI component of the laboratory analysis. LG, AC and AS extracted the clinical data. LG performed the data analysis, and ZA, BM-M, RG and MM contributed to data interpretation. LG wrote the initial draft and ZA revised the paper. All authors reviewed the manuscript.

## Sponsors

Liverpool Women's Hospital was the study sponsor for this research.

## Competing interests:

All authors have completed the Unified Competing Interest form and declare: AC has received salary and study support grants from Wellbeing of Women charity for this research; LG has received study support grants from Wellbeing of Women charity for this research; JH, AS, JI, AA, BM-M and ZA were also members of the University of Liverpool during the grant from Wellbeing of Women; MM and RG received a Centre of Research Excellence grant from the Australian National Health and Medical Research Council for this work; MM and RG served on the board of Trajan Nutrition; RG holds a patent on stabilizing and analysing fatty acids; no other financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

## Consent

All participants provided written informed consent

## Table/Figure List

### Figure 1

Participant selection.

### Figure 2

Visualisation of the relationship between long-chain omega-3 fatty acid levels and pregnancy outcome in women with a previous sPTB/PPROM $<34^{+0}$ weeks.

### Table 1

Demographic details of the study population.

### Table 2

Normal distribution of plasma long-chain omega-3 fatty acids in the low-risk population sample.

## Table 3

Relationship between quintile of long-chain omega-3 (as defined by the low-risk population group) and pregnancy outcome in the high-risk group.

## Table 4

Relationship between quintile of fatty acids (as defined by Olsen et al[11]) and pregnancy outcome.

## Supplementary Table 1

Plasma long-chain Omega-3 fatty acid levels compared by pregnancy group and number of freeze:thaw cycles prior to sample analysis.

## Supplementary Table 2

Relationship between quintile of long-chain omega-3 (as defined by the low-risk population sample) and pregnancy outcome in the high-risk group using only samples without prior freeze:thaw cycles.

## Supplementary Table 3

Relationship between quintile of long-chain omega-3 (as defined by the low-risk population sample) and pregnancy outcome in the high-risk group using only samples with prior freeze:thaw cycles.



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# Figure and Table legends

## Figure 1

Participant selection. Two obstetric populations were used to recruit women. The first was women at high-risk of sPTB based on their history of previous sPTB. The second population was used to represent "normality" and consisted of women with a history of term birth only. Two stages of analysis were performed. The first was to visualise the relationship between long-chain omega-3 status and the occurrence of sPTB or PPROM under 34 weeks in the high-risk cohort. The second analysis used aetiological modelling to assess the contribution of long-chain omega-3 to recurrent early preterm birth, for this analysis a clear 'split' in the preterm and term cases was desired, and so births $34^{+0}$-$36^{+6}$ weeks were excluded from this analysis. 'Cases' consisted of women with recurrent sPTB or PPROM $<34^{+0}$ weeks. sPTB= Spontaneous Preterm Birth, PPROM=Preterm Prelabour Rupture of Membranes

## Figure 2:

Visualisation of the relationship between long-chain omega-3 fatty acid levels and pregnancy outcome in women with a previous sPTB/PPROM $<34^{+0}$ weeks. Total n=283, of whom 51 had recurrent sPTB/PPROM, and the remainder (n=232) delivered $\geq 34^{+0}$ weeks without PPROM. A-D, histograms showing long-chain omega-3 fatty acid levels by pregnancy outcome. E-H risk of recurrent early sPTB/PPROM $<34^{+0}$ weeks by baseline fatty acid status in women with previous early sPTB or PPROM (n=283). The pale grey lines represent the 95% confidence interval for the risk. *P* values are for the association between long chain omega-3 status and risk of early preterm birth using fractional polymonial logistic regression. Graphs show the unadjusted data. Adjusted *P* values include the covariates of age, BMI, smoking and Index of Multiple Deprivation (IMD). Percentages are of the total plasma fatty acids.

## Table 1

Demographic details of the study population. BMI= Body Mass index, $kg/m^2$. IMD= Index of Multiple Deprivation. *P* value calculated by ANOVA for age, Kruskal-Wallis for BMI and Fisher's exact test for remainder of analysis. There were 3 missing values for BMI in the high-risk sPTB or PPROM <34 weeks group and 3 missing values in the high-risk birth ≥37 weeks term group. There was 1 missing value for smoking in the high-risk sPTB or PPROM

group and 3 missing values in the birth ≥37 weeks group. The IMD data section details the amount of data available, and all other data sections were complete. The high percentage of missing data for IMD was because postcode wasn't recorded for the high-risk group at the start of the research study.

## Table 2

Normal distribution of plasma long-chain omega-3 fatty acids in the low-risk population sample. Women were parous, with all previous births at term and birth ≥ 39+0 weeks in the index pregnancy. DHA=Docosahexaenoic acid, EPA=Eicosapentaenoic acid. All values are percentage of the total plasma fatty acids.

## Table 3

Relationship between quintile of long-chain omega-3 (as defined by the low-risk population sample) and pregnancy outcome in the high-risk group.. *=adjusted for age, BMI, and smoking (actual data only), **= adjusted for age, BMI, smoking and index of multiple deprivation (actual data only) ***= adjusted for age, BMI, smoking and index of multiple deprivation including imputed data for missing data.

## Table 4

Relationship between quintile of fatty acids (as defined by Olsen et al[11]) and pregnancy outcome. *=adjusted for age, BMI, and smoking (actual data only), **= adjusted for age, BMI, smoking and index of multiple deprivation (actual data only) ***= adjusted for age, BMI, smoking and index of multiple deprivation including imputed data for missing data.

## Supplementary Table 1:

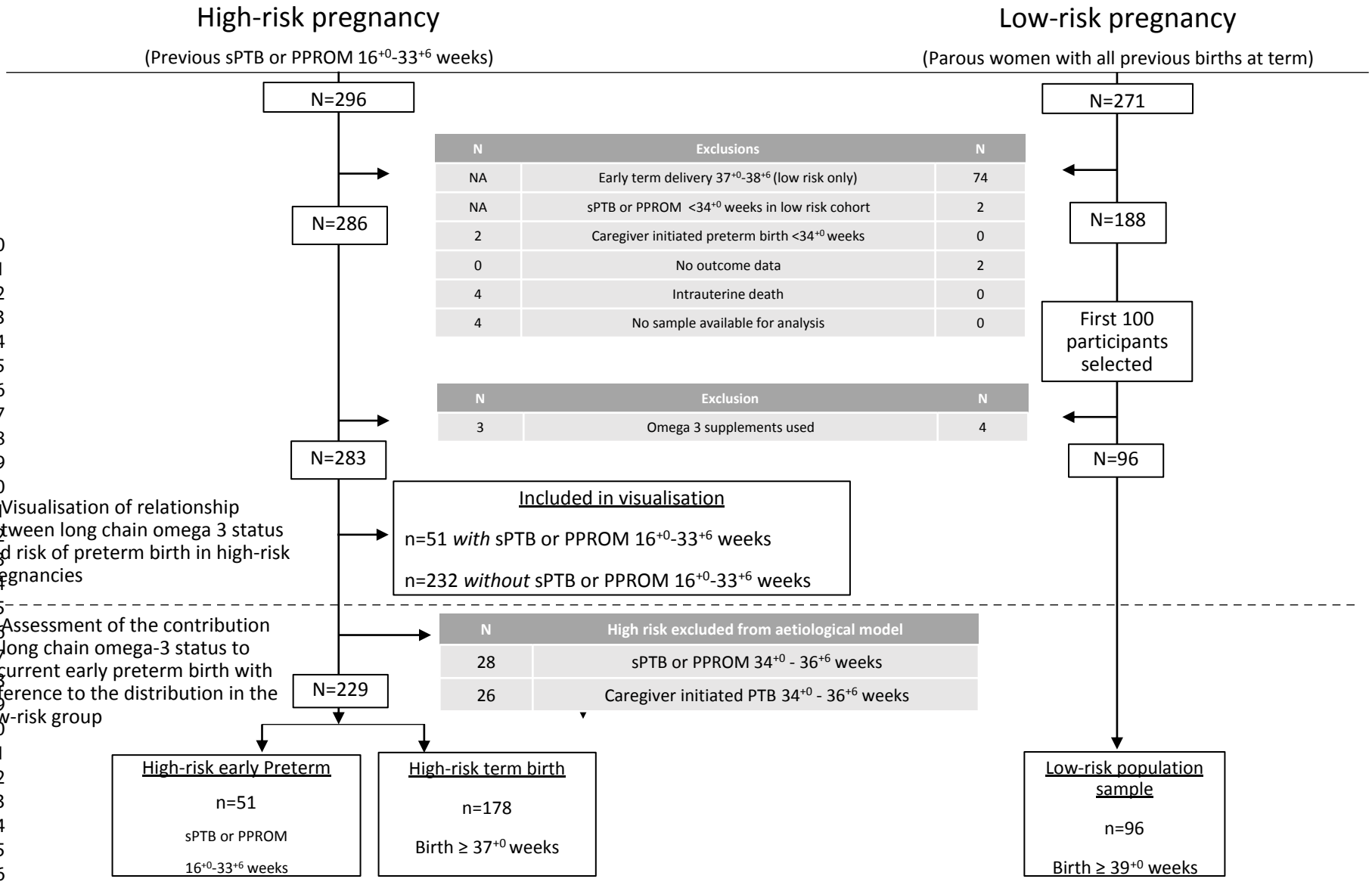
Plasma long-chain Omega-3 fatty acid levels compared by pregnancy group and number of freeze:thaw cycles prior to sample analysis.

## Supplementary Table 2:

Relationship between quintile of long-chain omega-3 (as defined by the low-risk population sample) and pregnancy outcome in the high-risk group using only samples without prior freeze:thaw cycles. *=adjusted for age, BMI, and smoking (actual data only).

## Supplementary Table 3:

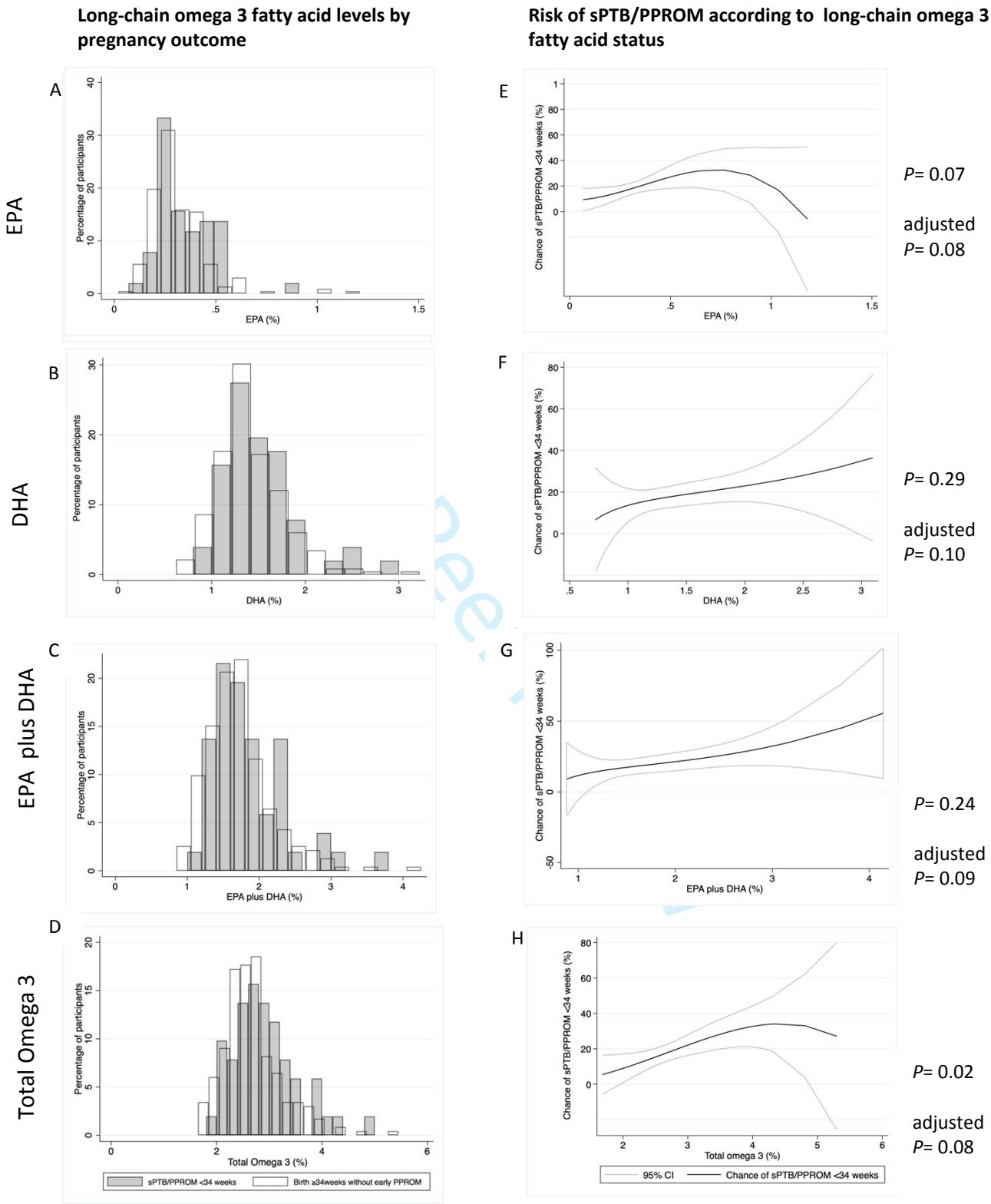
Relationship between quintile of long-chain omega-3 (as defined by the low-risk population sample) and pregnancy outcome in the high-risk group using only samples with prior freeze:thaw cycles. *=adjusted for age, BMI, and smoking (actual data only).




1) Visualisation of relationship between long chain omega 3 status and risk of preterm birth in high-risk pregnancies

2) Assessment of the contribution of long chain omega-3 status to recurrent early preterm birth with reference to the distribution in the low-risk group

Figure 2


Long-chain omega 3 fatty acid levels by pregnancy outcome
Risk of sPTB/PPROM according to long-chain omega 3 fatty acid status
EPA
DHA
EPA plus DHA
Total Omega 3
A
B
C
D
E
F
G
H
Percentage of participants
EPA (%)
DHA (%)
EPA plus DHA (%)
Total Omega 3 (%)
Chance of sPTB/PPROM <34 weeks (%)
sPTB/PPROM <34 weeks
Birth ≥34weeks without early PPROM
95% CI
Chance of sPTB/PPROM <34 weeks
P= 0.07
adjusted P= 0.08
P= 0.29
adjusted P= 0.10
P= 0.24
adjusted P= 0.09
P= 0.02
adjusted P= 0.08

# Table 1

| | | High-risk (previous sPTB or PPROM 16-33+6 weeks) | | | | | | Low-risk (previous term births) | | *P* value HR term vs HR preterm | *P* value LR vs HR participants of nested case-contol study |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Whole cohort** | | **Birth ≥37 weeks (term)** | | **sPTB/ PPROM <34 weeks (preterm)** | | **Birth ≥ 39 weeks** | | | |
| Purpose | | Visualisation | | Nested case-control study | | | | Define expected distribution of omega 3 fatty-acid levels | | | |
| | | n | SD/IQR/% | n | SD/IQR/% | n | SD/IQR/% | n | SD/IQR/% | | |
| n | | 283 | | 178 | | 51 | | 96 | | | |
| Age (mean, SD) | | 30.4 | 5.1 | 30.6 | 5.1 | 30.8 | 5 | 31.2 | 4.2 | 0.829 | 0.375 |
| BMI (median, IQR) | | 24.6 | 21.8-28.7 | 25.0 | 21.8-28.9 | 25.6 | 23.1-32.5 | 23.6 | 21.9-27.6 | 0.208 | 0.209 |
| Smoking (%) | | 68 | 24.0 | 37 | 20.9 | 13.0 | 26.5 | 9 | 9.7 | 0.438 | 0.011 |
| IMD quintile (%) | 1 (most deprived) | 127 | 67.2 | 82 | 67.2 | 21 | 75.0 | 47 | 49.4 | 0.757 | 0.011 |
| | 2 | 18 | 9.5 | 9 | 7.3 | 3 | 10.7 | 15 | 15.8 | | |
| | 3 | 19 | 10.1 | 11 | 9.0 | 2 | 7.1 | 19 | 20.0 | | |
| | 4 | 17 | 9.0 | 13 | 10.7 | 2 | 7.1 | 11 | 11.6 | | |
| | 5 (least deprived) | 8 | 4.2 | 7 | 5.7 | 0 | 0 | 3 | 3.2 | | |
| | Number of participants included in IMD data | 189 | 66.8 | 122 | 68.5 | 28 | 54.9 | 95 | 99.0 | | |
| Total number of previous sPTB or PPROM (%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 100 | <0.000 | not applicable |
| | 1 | 242 | 85.5 | 163 | 91.6 | 37 | 72.5 | | | | |
| | ≥ 2 | 41 | 14.5 | 15 | 8.4 | 14 | 27.4 | | | | |
| Previous cervical surgery | None | 254 | 89.8 | 167 | 93.8 | 42 | 82.4 | 88 | 91.7 | 0.094 | 0.395 |
| | ≤1x LLETZ <10mm | 19 | 6.7 | 9 | 5.1 | 6 | 11.8 | 8 | 8.3 | | |
| | 1x LLETZ ≥10mm/≥2 LLETZ/knife cone biopsy | 10 | 3.5 | 2 | 1.1 | 3 | 5.9 | | | | |
| Preterm birth prevention treatment used (%) | No | 190 | 67.1 | 128 | 71.9 | 27 | 52.9 | 96 | 100 | 0.017 | not applicable |
| | Yes | 93 | 32.9 | 50 | 28.1 | 24 | 47.1 | | | | |
| Gestational age at birth in weeks + days (median, IQR) | | 37+5 | 35+3-39+1 | 38+6 | 38+0-39+6 | 31+2 | 26+1-33+2 | 40+2 | 39+3-41+0 | not applicable | |
| Birthweight in grams (mean, SD) | | 2800 | 809 | 3238 | 477 | 1576 | 703 | 3614 | 439 | | |
| Preterm prelabour rupture of membranes <34 weeks (n, %) | | 21 | 7.42 | 0 | 0 | 21 | 46 | 0 | 0 | | |

# Table 2

| Component of fatty acids | Percentage of total fatty acids in low risk population sample (previous term births), birth ≥39 weeks in index pregnancy n=96 | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mean | (SD) | Range | Percentiles | | | |
| | | | | 20th | 40th | 60th | 80th |
| Total saturates | 36.9 | (2.36) | 29.9-42.8 | 35.2 | 36.2 | 36.9 | 38.8 |
| Total monosaturates | 29.3 | (2.91) | 21.4-37.5 | 27.0 | 28.8 | 30.2 | 31.6 |
| Omega 6 | 30.8 | (3.50) | 21.4-39.7 | 27.4 | 30.2 | 31.8 | 33.6 |
| Omega 3 | 2.65 | (0.49) | 1.87-4.09 | 2.18 | 2.51 | 2.74 | 3.07 |
| EPA | 0.32 | (0.12) | 0.14-0.77 | 0.22 | 0.27 | 0.31 | 0.37 |
| DHA | 1.35 | (0.32) | 0.73-2.14 | 1.10 | 1.22 | 1.38 | 1.62 |
| EPA +DHA | 1.67 | (0.39) | 0.95-2.88 | 1.36 | 1.51 | 1.70 | 1.98 |
| Arachidonic acid | 4.83 | (0.9) | 2.79-7.03 | 4.16 | 4.51 | 5.07 | 5.51 |

| | | Quintile | Level (%) | Total number of high risk participants | High-risk (previous sPTB or PPROM 16-33+6 weeks) | | | | Crude OR of PTB compared to quintile 3-5 within high risk (n=229) | | | Adjusted* OR compared to quintile 3-5 within high risk (n=220) | | | Adjusted** OR compared to quintile 3-5 within high risk (n=146) | | | Adjusted*** OR compared to quintile 3-5 within high risk (n=229) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Recurrent early sPTB/PPROM | | Birth ≥37 weeks | | OR of early sPTB/PPROM (95% CI) | 95%CI | *P*- value | OR of early sPTB/PPROM (95% CI) | 95%CI | *P*- value | OR of early sPTB/PPROM (95% CI) | 95%CI | *P*- value | OR of early sPTB/PPROM (95% CI) | 95%CI | *P*- value |
| | | | | | n | % | n | % | | | | | | | | | | | | |
| Quintiles based on low-risk population sample | Total Omega 3 | 1 | 1.69-2.18 | 27 | 5 | 9.8 | 22 | 12.4 | 0.65 | 0.23-1.84 | 0.42 | 0.63 | 0.21-1.85 | 0.40 | 0.83 | 0.23-2.98 | 0.77 | 0.51 | 0.14-1.78 | 0.29 |
| | | 2 | 2.18-2.51 | 59 | 9 | 17.6 | 50 | 28.1 | 0.52 | 0.23-1.15 | 0.11 | 0.58 | 0.25-1.33 | 0.20 | 0.86 | 0.30-2.52 | 0.79 | 0.48 | 0.18-1.24 | 0.13 |
| | | 3-5 | 2.51-5.29 | 143 | 37 | 72.5 | 106 | 59.6 | 1 | 1 | - | 1 | 1 | - | 1 | 1 | - | 1 | 1 | - |
| | EPA plus DHA | 1 | 0.88-1.36 | 37 | 8 | 15.7 | 29 | 16.3 | 0.91 | 0.38-2.15 | 0.83 | 1.04 | 0.43-2.54 | 0.93 | 0.88 | 0.26-2.98 | 0.84 | 0.75 | 0.27-2.10 | 0.58 |
| | | 2 | 1.36-1.51 | 29 | 5 | 9.8 | 24 | 13.5 | 0.69 | 0.24-1.91 | 0.47 | 0.69 | 0.24-2.01 | 0.50 | 1.64 | 0.49-5.55 | 0.42 | 0.60 | 0.18-2.03 | 0.41 |
| | | 3-5 | 1.51-4.1 | 163 | 38 | 74.5 | 125 | 70.2 | 1 | 1 | - | | | | | | | 1 | 1 | - |
| | DHA | 1 | 0.72-1.1 | 32 | 7 | 13.7 | 25 | 14.0 | 0.93 | 0.37-2.30 | 0.87 | 1.07 | 0.42-2.73 | 0.89 | 0.71 | 0.18-2.75 | 0.62 | 0.73 | 0.24-2.18 | 0.57 |
| | | 2 | 1.10-1.22 | 29 | 5 | 9.8 | 24 | 13.5 | 0.69 | 0.24-1.93 | 0.48 | 0.76 | 0.27-2.16 | 0.60 | 1.31 | 0.41-4.17 | 0.64 | 0.82 | 0.26-2.63 | 0.74 |
| | | 3-5 | 1.22-3.10 | 168 | 39 | 76.5 | 129 | 72.5 | 1 | 1 | - | 1 | 1 | - | 1 | 1 | - | 1 | 1 | - |
| | EPA | 1 | 0.07-0.22 | 53 | 8 | 15.7 | 45 | 25.3 | 0.53 | 0.22-1.24 | 0.14 | 0.55 | 0.23-1.32 | 0.18 | 0.45 | 0.13-1.57 | 0.21 | 0.35 | 0.12-1.03 | 0.06 |
| | | 2 | 0.22-0.27 | 53 | 12 | 23.5 | 41 | 23.0 | 0.87 | 0.41-1.86 | 0.72 | 0.83 | 0.37-1.85 | 0.64 | 0.61 | 0.21-1.77 | 0.36 | 0.57 | 0.22-1.48 | 0.25 |
| | | 3-5 | 0.27-1.18 | 123 | 31 | 60.8 | 92 | 51.7 | 1 | 1 | - | 1 | 1 | - | 1 | 1 | - | 1 | 1 | - |
| | Total | | | 229 | 51 | 100 | 178 | 100 | | | | | | | | | | | | |

# Table 4

| EPA plus DHA as per Olsen 2018 | | Low-risk (previous term births) | | High-risk (previous sPTB or PPROM 16-33+6 weeks) | | | | Crude OR of PTB compared to quintile 3-5 within high risk (n=229) | | | Adjusted* OR compared to quintile 3-5 within high risk (n=220) | | | Adjusted** OR compared to quintile 3-5 within high risk (n=146) | | | Adjusted*** OR compared to quintile 3-5 within high risk (n=229) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Quintile** | **Value** | **Birth ≥39 weeks** | | **Recurrent early sPTB/PPROM** | | **Birth ≥37 weeks** | | OR of early sPTB/PPROM (95% CI) | 95%CI | *P* - value | OR of early sPTB/PPROM (95% CI) | 95%CI | *P* - value | OR of early sPTB/PPROM (95% CI) | 95%CI | *P* - value | OR of early sPTB/PPROM (95% CI) | 95%CI | *P* - value |
| | | **n** | **%** | **n** | **%** | **n** | **%** | | | | | | | | | | | | |
| 1 | 0.47-1.42 | 25 | 26.0 | 8 | 15.7 | 40 | 22.5 | 0.62 | 0.26-1.51 | 0.30 | 0.72 | 0.29-1.78 | 0.48 | 0.58 | 0.17-2.04 | 0.40 | 0.61 | 0.25-1.51 | 0.29 |
| 2 | 1.43-1.74 | 38 | 39.6 | 18 | 35.3 | 60 | 33.7 | 0.94 | 0.47-1.87 | 0.852 | 0.97 | 0.47-2.03 | 0.94 | 1.52 | 0.57-4.04 | 0.40 | 0.87 | 0.42-1.79 | 0.71 |
| 3-5 | 1.74-4.95 | 33 | 34.4 | 25 | 49.0 | 78 | 43.8 | 1 | 1 | - | 1 | 1 | - | 1 | 1 | - | 1 | 1 | - |
| | Total | 96 | 100 | 51 | 100 | 178 | 100 | | | | | | | | | | | | |

| | Low-risk term | | | | | High-risk term | | | | | High-risk early sPTB/PPROM | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 freeze thaw cycles n=80 | | 3 freeze thaw cycles n=16 | | Mann-Whitney p | 0 freeze thaw cycles n=162 | | 3 freeze thaw cycles n=16 | | Mann-Whitney *P* | 0 freeze thaw cycles n=22 | | 3 freeze thaw cycles n=29 | | Mann-Whitney *P* |
| | median | IQR | median | IQR | | median | IQR | median | IQR | | median | IQR | median | IQR | |
| DHA | 1.34 | 0.43 | 1.27 | 0.32 | 0.81 | 1.39 | 0.42 | 1.40 | 0.57 | 0.96 | 1.42 | 0.48 | 1.40 | 0.50 | 0.63 |
| EPA | 0.29 | 0.13 | 0.30 | 0.09 | 0.51 | 0.28 | 0.15 | 0.26 | 0.16 | 0.52 | 0.33 | 0.16 | 0.27 | 0.19 | 0.17 |
| DHA+EPA | 1.61 | 0.53 | 1.64 | 0.41 | 0.98 | 1.69 | 0.49 | 1.63 | 0.61 | 0.73 | 1.83 | 0.61 | 1.70 | 0.53 | 0.57 |

*Table S1: Plasma long-chain Omega 3 fatty acid levels compared by pregnancy group and number of freeze:thaw cycles prior to sample analysis.*

| Basis for quintiles | | Quintile | Level (%) | Total number of high-risk participants | High-risk (previous sPTB or PPROM 16-33+6 weeks) | | | | Crude OR of PTB compared to quintile 3-5 within high risk (n=45) | | | Adjusted* OR compared to quintile 3-5 within high risk (n=44) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Recurrent early sPTB or PPROM <34 weeks | | Birth ≥37 weeks | | OR of early sPTB/PPROM (95% CI) n=184 | 95%CI | *P*- value | OR of early sPTB/PPROM (95% CI) n=184 | 95%CI | *P*- value |
| | | | | | n | % | n | % | | | | | | |
| Low-risk population sample | **Total Omega 3** | 1 | 1.69-2.18 | 22 | 2 | 9.1 | 20 | 12.3 | 0.66 | 0.14-3.11 | 0.60 | 0.74 | 0.15-3.67 | 0.71 |
| | | 2 | 2.18-2.51 | 48 | 5 | 22.7 | 43 | 26.5 | 0.77 | 0.26-2.24 | 0.63 | 0.98 | 0.32-3.02 | 0.98 |
| | | 3-5 | 2.51-5.29 | 114 | 15 | 68.2 | 99 | 61.1 | 1 | 1 | - | 1 | 1 | - |
| | **EPA plus DHA** | 1 | 0.88-1.36 | 28 | 2 | 9.1 | 26 | 16.0 | 0.52 | 0.11-2.39 | 0.40 | 0.60 | 0.13-2.84 | 0.52 |
| | | 2 | 1.36-1.51 | 24 | 3 | 13.6 | 21 | 13.0 | 0.97 | 0.26-3.59 | 0.96 | 1.11 | 0.29-4.31 | 0.88 |
| | | 3-5 | 1.51-4.1 | 132 | 17 | 77.3 | 115 | 71.0 | 1 | 1 | - | 1 | 1 | - |
| | **DHA** | 1 | 0.72-1.1 | 24 | 2 | 9.1 | 22 | 13.6 | 0.63 | 0.14-2.93 | 0.56 | 0.74 | 0.15-3.57 | 0.71 |
| | | 2 | 1.10-1.22 | 25 | 3 | 13.6 | 22 | 13.6 | 0.95 | 0.26-3.50 | 0.93 | 1.07 | 0.28-4.07 | 0.92 |
| | | 3-5 | 1.22-3.10 | 135 | 17 | 77.3 | 118 | 72.8 | 1 | 1 | - | 1 | 1 | - |
| | **EPA** | 1 | 0.07-0.22 | 42 | 2 | 9.1 | 40 | 24.7 | 0.25 | 0.06-1.13 | 0.07 | 0.29 | 0.06-1.35 | 0.11 |
| | | 2 | 0.22-0.27 | 40 | 3 | 13.6 | 37 | 22.8 | 0.41 | 0.11-1.47 | 0.17 | 0.46 | 0.12-1.72 | 0.25 |
| | | 3-5 | 0.27-1.18 | 102 | 17 | 77.3 | 85 | 52.5 | 1 | 1 | - | 1 | 1 | - |
| Olsen *et al.* | **EPA plus DHA** | 1 | 0.47-1.42 | 39 | 2 | 9.1 | 37 | 1.2 | 0.32 | 0.07-1.53 | 0.15 | 0.38 | 0.08-1.84 | 0.23 |
| | | 2 | 1.43-1.74 | 61 | 8 | 36.4 | 53 | 4.9 | 0.91 | 0.35-2.37 | 0.84 | 0.95 | 0.33-2.74 | 0.93 |
| | | 3-5 | 1.74-4.95 | 84 | 12 | 54.5 | 72 | 7.4 | 1 | 1 | - | 1 | 1 | - |
| | | | Total | 184 | 22 | | 162 | | | | | | | |

*Table S2: Relationship between quintile of long-chain omega 3 (as defined by the low-risk reference group) and pregnancy outcome in the high-risk group using only samples without prior freeze:thaw cycles. *=adjusted for age, BMI, and smoking (actual data only).*

| Basis for quintiles | | Quintile | Level (%) | Total number of high risk participants | High-risk (previous sPTB or PPROM 16-33+6 weeks) | | | | Crude OR of PTB compared to quintile 3-5 within high risk (n=45) | | | Adjusted* OR compared to quintile 3-5 within high risk (n=44) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Recurrent early sPTB/PPROM | | Birth ≥37 weeks | | OR of early sPTB/PPROM (95% CI) | 95%CI | *P* - value | OR of early sPTB/PPROM (95% CI) | 95%CI | *P* - value |
| | | | | | n | % | n | % | | | | | | |
| Low-risk population sample | **Total Omega 3** | 1 | 1.69-2.18 | 5 | 3 | 10.3 | 2 | 12.5 | 0.48 | 0.07-3.46 | 0.46 | 0.43 | 0.05-3.50 | 0.43 |
| | | 2 | 2.18-2.51 | 11 | 4 | 13.8 | 7 | 43.8 | 0.18 | 0.04-0.81 | 0.03 | 0.18 | 0.03-0.96 | 0.05 |
| | | 3-5 | 2.51-5.29 | 29 | 22 | 75.9 | 7 | 43.8 | 1 | 1 | - | 1 | 1 | - |
| | **EPA plus DHA** | 1 | 0.88-1.36 | 9 | 6 | 20.7 | 3 | 18.8 | 0.95 | 0.20-4.61 | 0.95 | 1.67 | 0.32-8.68 | 0.55 |
| | | 2 | 1.36-1.51 | 5 | 2 | 6.9 | 3 | 18.8 | 0.32 | 0.046-2.21 | 0.25 | 0.44 | 0.04-4.57 | 0.50 |
| | | 3-5 | 1.51-4.1 | 31 | 21 | 72.4 | 10 | 62.5 | 1 | 1 | - | 1 | 1 | - |
| | **DHA** | 1 | 0.72-1.1 | 8 | 5 | 17.2 | 3 | 18.8 | 0.83 | 0.16-4.14 | 0.82 | 1.29 | 0.24-7.05 | 0.77 |
| | | 2 | 1.10-1.22 | 4 | 2 | 6.9 | 2 | 12.5 | 0.50 | 0.06-4.03 | 0.52 | 0.69 | 0.07-7.00 | 0.75 |
| | | 3-5 | 1.22-3.10 | 33 | 22 | 75.9 | 11 | 68.8 | 1 | 1 | - | 1 | 1 | - |
| | **EPA** | 1 | 0.07-0.22 | 11 | 6 | 20.7 | 5 | 31.3 | 0.60 | 0.13-2.67 | 0.50 | 0.72 | 0.14-3.59 | 0.69 |
| | | 2 | 0.22-0.27 | 13 | 9 | 31.0 | 4 | 25.0 | 1.13 | 0.25-4.98 | 0.88 | 0.99 | 0.19-5.10 | 0.99 |
| | | 3-5 | 0.27-1.18 | 21 | 14 | 48.3 | 7 | 43.8 | 1 | 1 | - | 1 | 1 | - |
| Olsen *et al.* | **EPA plus DHA** | 1 | 0.47-1.42 | 9 | 6 | 10.3 | 3 | 18.8 | 0.92 | 0.17-5.00 | 0.93 | 1.43 | 0.25-8.27 | 0.69 |
| | | 2 | 1.43-1.74 | 17 | 10 | 24.1 | 7 | 43.8 | 0.66 | 0.17-2.59 | 0.55 | 0.53 | 0.11-2.46 | 0.42 |
| | | 3-5 | 1.74-4.95 | 19 | 13 | 20.7 | 6 | 37.5 | 1 | 1 | - | 1 | 1 | - |
| | | | Total | 45 | 29 | | 16 | | | | | | | |

*Table S3: Relationship between quintile of long-chain omega 3 (as defined by the low-risk reference group) and pregnancy outcome in the high-risk group using only samples with prior freeze:thaw cycles. *=adjusted for age, BMI, and smoking (actual data only).*